PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 07 November 2000 (07.11.00)	
International application No. PCT/CA00/00289	**Applicant's or agent's file reference** 1038-1025
International filing date (day/month/year) 16 March 2000 (16.03.00)	**Priority date** (day/month/year) 16 March 1999 (16.03.99)
Applicant LOOSMORE, Sheena, M. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

11 October 2000 (11.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
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Authorized officer

Nestor Santesso

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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION THAT INTERNATIONAL APPLICATION CONSIDERED TO BE WITHDRAWN

(PCT Article 14(1), (3) or (4) and Rule 29.1)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE
in its capacity as designated Office

Date of mailing (day/month/year)	**IMPORTANT NOTIFICATION**
28 August 2001 (28.08.01)	
International application No.	**International filing date** (day/month/year)
PCT/CA00/00289	16 March 2000 (16.03.00)
Applicant	
CONNAUGHT LABORATORIES LIMITED et al	

1. The International Bureau hereby gives notice that the receiving Office has, on the date indicated below, notified to the applicant that the international application is to be considered withdrawn:

   01 June 2001 (01.06.01)

2. [X] A copy of this notification has been sent to the International Preliminary Examining Authority.

The International Bureau of WIPO
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09/936,362

PATENT COOPERATION TREATY

REC'D 19 JUL 2001
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1038-1025		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00289	16/03/2000	16/03/1999
International Patent Classification (IPC) or national classification and IPC C07K14/00		
Applicant CONNAUGHT LABORATORIES LIMITED et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11/10/2000	17.07.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Zellner, E Telephone No. +49 89 2399 8427

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00289

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-13,16,17, 19-63	as originally filed			
14,15,18	as received on	22/06/2001	with letter of	22/06/2001

Claims, No.:

1-29	as received on	22/06/2001	with letter of	22/06/2001

Drawings, sheets:

1/83-83/83	as originally filed

Sequence listing part of the description, pages:

2-75, filed with the letter of 29.05.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-2,4-29
	No:	Claims 3
Inventive step (IS)	Yes:	Claims 4
	No:	Claims 1-3,5-29
Industrial applicability (IA)	Yes:	Claims 1-29
	No:	Claims

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

D1: WO 96 30519 A (UNIV WASHINGTON ;UNIV ST LOUIS (US); ST GEME JOSEPH W III (US); BA) 3 October 1996 (1996-10-03)

D2: GEME J W S ET AL: 'CHARACTERIZATION OF THE GENETIC LOCUS ENCODING HAEMOPHILUS INFLUENZAE TYPE B SURFACE FIBRILS' JOURNAL OF BACTERIOLOGY,US,WASHINGTON, DC, vol. 178, no. 21, November 1996 (1996-11), pages 6281-6287, XP000863110 ISSN: 0021- 9193

D3: BARENKAMP S J ET AL: 'IDENTIFICATION OF A SECOND FAMILY OF HIGH-MOLECULAR-WEIGHT ADHESION PROTEINS EXPRESSED BY NON-TYPABLE HAEMOPHILUS INFLUENZAE' MOLECULAR MICROBIOLOGY,GB,BLACKWELL SCIENTIFIC, OXFORD, vol. 19, no. 6, 1996, pages 1215-1223, XP000579265 ISSN: 0950-382X

Item I

The amendment of Claim 3 is not allowable under Articles 19(2) and 34 (2) (b) PCT. Additional feature "N-truncated protein having the ability to bind to human epithelial cells" is not disclosed in the description as originally filed. For the N-truncated hia proteins it is only described that immunization causes protection against colonization (see Examples).

Item IV

The present set of claims are not linked in manner so as to form a single general inventive concept as required under Rule 13(1) PCT.
The problem underlying the invention of the present application is the provision of a set of nucleotide and amino acid sequences of adhesion (Hia) from non-typeable strains of Haemophilus influenzae.

The solution is represented by the set of amino- and nucleic acid sequences as set forth in SEQ. ID. Nos 23-36.

The international patent application WO9630519 discloses adhesins from non-typeable strains of Haemophilus influenzae, as well as methods for their recombinant production

and their use in immunogenic compositions and production of antibodies (see abstract, example 3, page 82-84).

Genes from non-typeable H. influenzae coding for Hia adhesins are also disclosed by St. Geme et al. in Infection and Immunity (1998, p. 364-368, see abstract).

Therefore the concept of DNA encoding adhesins from non typeable H. influenzae is not new. In consequence, the different adhesins of the present application fall a posteriori into 6 groups of alleged inventions.

1. Claims 1-27 (partially)
   An isolated and purified nucleic acid molecule having a sequence as set forth in SEQ ID NO. 23 encoding an polypeptide of a Haemophilus influenzae adhesion having the primary structure as set forth in SEQ ID NO. 24. Vectors for the recombinant production of said adhesion, immunogenic compositions containing the same.

2. An isolated and purified nucleic acid molecule having a sequence as set forth in SEQ ID NO. 27 encoding an polypeptide of a Haemophilus influenzae adhesion having the primary structure as set forth in SEQ ID NO. 28. Vectors for the recombinant production of said adhesion, immunogenic compositions containing the same.

3. An isolated and purified nucleic acid molecule having a sequence as set forth in SEQ ID NO. 29 encoding an polypeptide of a Haemophilus influenzae adhesion having the primary structure as set forth in SEQ ID NO. 30. Vectors for the recombinant production of said adhesion, immunogenic compositions containing the same.

4. An isolated and purified nucleic acid molecule having a sequence as set forth in SEQ ID NO. 31 encoding an polypeptide of a Haemophilus influenzae adhesion having the primary structure as set forth in SEQ ID NO. 32. Vectors for the recombinant production of said adhesion, immunogenic compositions containing the same.

5. An isolated and purified nucleic acid molecule having a sequence as set forth in SEQ ID NO. 33 encoding an polypeptide of a Haemophilus influenzae adhesion having the primary structure as set forth in SEQ ID NO. 34. Vectors for the recombinant production of said adhesion, immunogenic compositions containing the same.

6. An isolated and purified nucleic acid molecule having a sequence as set forth in SEQ ID NO. 35 encoding an polypeptide of a Haemophilus influenzae adhesion having the primary structure as set forth in SEQ ID NO. 36. Vectors for the recombinant production of said adhesion, immunogenic compositions containing the same.

Item V

1. Novelty:

Claim 3 is not allowable under Article 33 (2) PCT.
Due to the generic and broad definition (especially the wording "truncated" and "expressible" of said claim (see also item VIII) all sorts of H. influenza adhesion encoding nucleotides fall under the definition of Claim 3.
In other words all adhesion nucleotides encoding for any adhesion being shorter (i.e. truncations of only one or two amino acids) than an adhesion of the present application lies within the definition such as those of D1 (see e.g. sequence comparisons of the Search Examiner page 6, in comparison with GSP:R99394 is shorter than no SEQ ID 28).

2. Inventive step

D1 is considered to represent the closest prior art document. D1 teaches Haemophilus adhesion proteins nucleic acids and derived vaccines. SEQ ID NO 36 of the present application has **97%** identity with the amino acid sequence of D1, SEQ ID NO 32 has **79%** identity with the amino acid sequence of D1 (Sequence Comparisons of the Search Examiner).
The problem of the present application is to provide further H. influenza adhesions

proteins and their encoding genes. As soon as one family member of the Haemophilus influenza adhesion protein, its gene the recombinant production and its immunological use is known, it is routine for a skilled person to determine further similar members from other strains of said proteins their immunogenic fragments and their encoding genes.
In this case the cloning and expression, although requiring much work, does not pose such problems so that there was no reasonable expectation of success.
For a skilled person it is also obvious to provide non-specified truncated versions of said genes or proteins having no particular unexpected effect (Claim 3).

In consequence, the present claims 1-3, 5-29 are not allowable under Article 33 (3) PCT.

The specific truncated Hia proteins of Claim 4 fulfil the requirements under Article 33(2) and (3) PCT.

The essential difference with D1 is the truncated form wherein the signal sequence is deleted causing a higher expression in E. coli. Said truncated proteins are still immunogenic (see Examples).
The problem of the present application can thus be defined as the provision of alternative hia proteins which can be produced recombinantly in a high amount still causing immunity.
The solution i.e. the truncated hia proteins of claim 4 are not derivable from D1 or any other document cited in the Search Report.

Item VIII

1. Claim 2 is formulated in terms of a "product by process". In the PCT contracting states no unified criteria exist concerning this type of claims. Before the EPO such claims, defined in terms of a product by process of manufacture are only admissible if the product as such fulfils the requirements of patentability, i.e. if the products are novel and inventive and if the product cannot be defined by true technical features (Article 6 PCT).

   The same applies to claim 15.

2. Independent Claim 3 does not disclose any true technical features. The only characteristic of the claimed nucleic acids is that they are "truncated" and "expressible as inclusion bodies". In consequence, said claim is vague and thus not clear (Article 6 PCT).

3. The Applicant should prove whether the strains of Claim 27 are known by the skilled person. Otherwise said claim is not clear.

generate the sites. Upperstrand (SEQ ID No.: 50) lower strand (SEQ ID No.: 51).

Figure 7A shows the construction of plasmids DS-2242-1 and DS-2242-2 that contain the T7 promoter and full-length NTHi strain 33 *hia* gene, the *E. coli cer* gene and the kanamycin resistance gene. Restriction enzyme sites are: A, *AlwN* I; B, *BamH* I; Bg, *Bgl* II; H, *Hind* III; K, *Kpn* I; N, *Nde* I; Ps, *Pst* I; R, *EcoR* I; S, *Sal* I; Sm, *Sma* I; Xb, *Xba* I; Xho, *Xho* I. Other abbreviations are: T7p, T7 promoter; ApR, ampicillin resistance; KanR, kanamycin resistance; tt1, transcription terminator 1 from *trpA*; tt2, transcription terminator 2 from T7 gene 10.

Figure 7B shows the oligonucleotides used to generate the 5'-end of the strain 33 *hia* gene coding strand (SEQ ID No.: 52), complementary strand (SEQ ID No.: 53), and encoded amino acid sequence (SEQ ID No.: 54).

Figure 8A shows the construction of plasmid DS-2340-2-3 that contains the T7 promoter and the V38 *hia* gene from strain 33, the *E. coli cer* gene and the kanamycin resistance gene. Restriction enzyme sites are: B, *BamH* I; Bg, *Bgl* II; H, *Hind* III; N, *Nde* I; Ps; *Pst* I; R, *EcoR* I; S, *Sal* I; Sn, *SnaB* I; Xb, *Xba* I. Other abbreviations are: T7p, T7 promoter; ApR, ampicillin resistance; KanR, kanamycin resistance; tt1, transcription terminator 1 from *trpA*; tt2, transcription terminator 2 from T7 gene 10.

Figure 8B shows the oligonucleotides used to PCR amplify the 5'-end of the truncated *hia* gene. Sense (6286.SL): SEQ ID No: 60, encoded amino acids SEQ ID

No: 61; antisense (6287.SL) SEQ ID No: 18, complement SEQ ID No: 19, encoded amino acids SEQ ID No: 20.

Figures 9A and 9B show the construction of plasmids DS-2447-2 and DS-2448-17, that contain tandem copies of the *T7 V38 hia (11)* and *T7 V38 hia (33)* genes, respectively. Restriction enzyme sites are: B, BamH I; Bg, Bgl II; H, Hind III; Ps; Pst I; R, EcoR I; S, Sal I; Xb, Xba I. Other abbreviations are: T7p, T7 promoter; ApR, ampicillin resistance; KanR, kanamycin resistance; CAP, calf alkaline phosphatase; tt1, transcription terminator 1 from *trpA*; tt2, transcription terminator 2 from T7 gene 10.

Figure 10 shows the expression of rHia. Panel A: lane 1, full-length rHia (11) no induction; lane 2, full-length rHia (11); lane 3, E21 rHia (11); lane 4, T33 rHia (11); lane 5, V38 rHia (11); lane 6, N52 rHia (11). Panel B: lane 1, V38 rHia (11) no induction; lane 2, V38 rHia (11); lane 3, V38 rHia (11)/cer.

Figure 11 shows a purification scheme for rHia proteins. Abbreviations are: SP, supernatant; PPT, precipitate; DTT, dithiothreitol; OG, octyl glucoside; (x) means discarded.

Figure 12, having panels A and B, shows the SDS-PAGE analysis of purified rHia. Panel A shows purified V38 rHia protein from strain 11 and panel B shows purified V38 rHia protein from strain 33. Lane 1, molecular weight markers; lane 2, whole-cell lysate; lane 3, crude extract; lane 4, purified rHia protein.

Figure 13, having panels A, B and C, shows the stability of V38 rHia (11). Panel A shows samples stored at 4°C without glycerol. Panel B shows samples

Moraxella catarrhalis high molecular weight proteins (200 kDa) from strains 4223 and LES-1 (SEQ ID Nos.: 48, 49). Asterisks within sequences indicate stop codons, but below the sequence they indicated sequence homology. Dots indicate identical residues. The sequence alignments were prepared by direct comparison of the amino acid sequences of the respective proteins.

Figure 29 shows the oligonucleotides used to PCR amplify the 5' end of the hia gene at the S44 truncated position. Sense (6817.SL) SEQ ID No: 55, encoding amino acids SEQ ID No: 56; antisense (6818.SL) SEQ ID No: 57, complement SEQ ID No: 58, encoded amino acids SEQ ID No: 59.

Figure 30 shows the construction of plasmid JB-2930-3 that contains the S44 *hia* gene from NTHi strain 11 and the E. coli *cer* gene and the T7 promoter. Restriction enzyme sites are: B, *BamH* I; Bg, *Bgl* II; K, *Kpn* I; N, *Nde* I; P, *Pst* I; R, *EcoR* I; S, *Sal* I; Sm, *Sma* I; Sty, *Sty* I; Xb, *Xba* I; Xho, *Xho* I. Other abbreviations are: T7p, T7 promoter; ApR, ampicillin resistance; KanR, kanamycin resistance; CAP, calf alkaline phosphatase; tt1 transcription terminator 1 from trpA; tt2, transcription terminator 2 from T7 gene 10.

Figure 31 shows SDS-PAGE analysis of the expression of rHia from S44. Lane 1, expression from pET S44 vector at time 0 (no induction); lane 2 expression from pET S44 vector after 4 hours induction; lane 3 expression from JB-2930-3 after 4 hours induction.

CLAIMS

1. An isolated and purified nucleic acid molecule encoding a *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* having:

(a) a DNA sequence selected from the group consisting of those shown in Figures 18, 20, 21, 22, 23, 24 and 25 (SEQ ID Nos: 23, 27, 29, 31, 33, 35, 37); or

(b) a DNA sequence encoding a *Haemophilus influenzae* adhesin (Hia) protein having an amino acid sequence selected from the group consisting of those shown in Figures 18, 20, 21, 22, 23, 24 and 25 (SEQ ID Nos: 24, 28, 30, 32, 34, 36, 38).

2. An isolated and purified nucleic acid molecule encoding an N-truncated *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* which is amplifiable by a pair of nucleotides which are selected from the group consisting of:

SEQ ID No: 7 and SEQ ID No: 15
SEQ ID No: 9 and SEQ ID No: 15
SEQ ID No: 11 and SEQ ID No: 15
SEQ ID No: 13 and SEQ ID No: 15
SEQ ID No: 55 and SEQ ID No: 57

3. An isolated and purified nucleic acid encoding an N-truncated *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* expressed as inclusion bodies, said N-truncated protein having the ability to bind to human epithelial cells.

4. The nucleic acid molecule of claim 3 which encodes a truncated Hia protein selected from the group consisting of the E21, T33, V38 and N52 truncations of *Haemophilus influenzae* strain 11 and the V38 truncation of *Haemophilus influenzae* strain 33.

5. A vector for transforming a host comprising the nucleic acid molecule of claim 1.

6. A vector for transforming a host comprising the nucleic acid molecule of any one of claims 2 to 4.

7. The vector of claim 5 or 6 which is a plasmid vector.

8. The vector of claim 7 wherein said plasmid vector has the identifying characteristics of a plasmid which is selected from the group consisting of:

DS-2008-2-3 as shown in Figure 1A

DS-2186-1-1 as shown in Figure 5A

DS-2201-1 as shown in Figure 5A

DS-2186-2-1 as shown in Figure 5A

DS-2188-2-6 as shown in Figure 5A

IA-191-3-1 as shown in Figure 32

9. A vector for transforming a host, comprising a nucleic acid molecule encoding a full-length *Haemophilus influenzae* adhesin (Hia) protein as claimed in claim 1 or N-truncated *Haemophilus influenzae* adhesin (Hia) protein as claimed in any one of claims 2 to 4 and a promoter operatively connected to said nucleic acid molecule for expression of said full-length or truncated Hia protein.

10. The vector of claim 9 further comprising the *cer* gene of *E. coli*.

11. The vector of claim 9 which is a plasmid vector.

12. The vector of claim 11 wherein said plasmid vector has the identifying characteristics of a plasmid vector which is selected from the group consisting of:

BK-96-2-11 as shown in Figure 6A

DS-2242-1 as shown in Figure 7A

DS-2242-2 as shown in Figure 7A

DS-2340-2-3 as shown in Figure 8A

DS-2447-2 as shown in Figure 9A

DS-2448-17 as shown in Figure 9B

JB-2930-3 as shown in Figure 32

13. A host cell transformed by a vector as claimed in claim 5, 6 or 9 and expressing a protective *Haemophilus influenzae* adhesin (Hia) protein of a non-typeable strain of *Haemophilus*.

14. The host cell of claim 13 which is a strain of *E. coli*.

15. A recombinant protective *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* producible by the transformed *E. coli* of claim 14 or an immunogenic fragment thereof.

16. An immunogenic composition, comprising at least one immunologically-active component selected from the group consisting of:

(A) an isolated and purified nucleic acid molecule encoding a *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* having:

(a) a DNA sequence selected from the group consisting of those shown in Figures 18, 20, 21, 22, 23, 24 and 25 (SEQ ID Nos: 23, 27, 29, 31, 33, 35, 37); or

(b) a DNA sequence encoding a *Haemophilus influenzae* adhesin (Hia) protein having an amino acid sequence selected from the group consisting of those shown in Figures 18, 20, 21, 22, 23, 24 and 25 (SEQ ID Nos: 24, 28, 30, 32, 34, 36, 38);

(B) an isolated and purified nucleic acid molecule encoding an N-truncated *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* which is amplifiable by a pair of nucleotides which are selected from the group consisting of:

SEQ ID No: 7 and SEQ ID No: 15
SEQ ID No: 9 and SEQ ID No: 15
SEQ ID No: 11 and SEQ ID No: 15
SEQ ID No: 13 and SEQ ID No: 15
SEQ ID No: 55 and SEQ ID No: 57;

(C) an isolated and purified nucleic acid molecule encoding a truncated *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* expressed as inclusion bodies, said N-truncated protein having the ability to bind to human epithelial cells; and

(D) a recombinant protective *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae* producible by a strain of *E. coli* transformed by an expression vector as claimed in claim 5, 6 or 9; and

a pharmaceutically-acceptable carrier therefor.

17. The immunogenic composition of claim 16 formulated as a vaccine for *in vivo* administration to protect against disease caused by *Haemophilus*.

18. The immunogenic composition of claim 16 in combination with a targeting molecule for delivery to specific cells of the immune system or to mucosal surfaces.

19. The immunogenic composition of claim 16 formulated as a microparticle, capsule or liposome preparation.

20. The immunogenic composition of claim 16 further comprising an adjuvant.

21. A method for inducing protection against disease caused by *Haemophilus*, comprising administering to a susceptible host an effective amount of the immunogenic composition of claim 16.

22. The method of claim 21 wherein the susceptible host is a human.

23. A method for the production of a protective *Haemophilus influenzae* adhesin (Hia) protein of a non-typeable strain of *Haemophilus influenzae*, which comprises:

transforming a host with a vector as claimed in claim 6,

growing the host cell to express the encoded truncated Hia, and

isolating and purifying the expressed Hia protein.

24. The method of claim 23 wherein the host cell is *E. coli*.

25. The method of claim 23 wherein said encoded truncated Hia is expressed in inclusion bodies.

26. The method of claim 25 wherein said isolation and purification of the expressed Hia is effected by:

disrupting the grown transformed cells to produce a supernatant and the inclusion bodies,

solubilizing the inclusion bodies to produce a solution of the recombinant Hia,

chromatographically purifying the solution of recombinant Hia free from cell debris, and

isolating the purified recombinant Hia protein.

27. The method of claim 23 wherein said non-typeable strain of *Haemophilus* is selected from the group consisting of strains 11, 33, 32, 29, M4071, K9, K22 and 12.

28. The method of claim 23 wherein said vector includes the T7 promoter and said *E. coli* is cultured in the presence of an inducing amount of lactose.

29. A pair of nucleotide sequences capable of amplifying and generating a nucleic acid molecule encoding an N-truncated *Haemophilus influenzae* adhesin (Hia) protein of a strain of *Haemophilus influenzae*, which pair of nucleotides is selected from the group consisting of:

SEQ ID No: 7 and SEQ ID No: 15

SEQ ID No: 9 and SEQ ID No: 15

SEQ ID No: 11 and SEQ ID No: 15

SEQ ID No: 13 and SEQ ID No: 15

SEQ ID No: 55 and SEQ ID No: 57

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

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21 September 2000 (21.09.2000)

PCT

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(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 09/268,347 (CIP)
Filed on 16 March 1999 (16.03.1999)

(71) Applicant *(for all designated States except US)*: **CONNAUGHT LABORATORIES LIMITED** [CA/CA]; 1755 Steeles Avenue, Toronto, Ontario M2R 3T4 (CA).

(72) Inventors; and
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(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
2 August 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/55191 A3

(54) Title: RECOMBINANT *HAEMOPHILUS INFLUENZAE* ADHESIN PROTEINS

(57) Abstract: Recombinant production of Hia protein, in full-length and N-terminally truncated forms, of non-typeable strains of *Haemophilus influenzae*, is described. The nucleic acid and deduced amino acid sequences of Hia genes of various strains of non-typeable and type c *Haemophilus influenzae* also are described.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00289

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/285 C12N15/00 A61K38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, WPI Data, EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 30519 A (UNIV WASHINGTON ;UNIV ST LOUIS (US); ST GEME JOSEPH W III (US); BA) 3 October 1996 (1996-10-03) abstract example 3 page 82 -page 84 ---	1-27
X	GEME J W S ET AL: "CHARACTERIZATION OF THE GENETIC LOCUS ENCODING HAEMOPHILUS INFLUENZAE TYPE B SURFACE FIBRILS" JOURNAL OF BACTERIOLOGY,US,WASHINGTON, DC, vol. 178, no. 21, November 1996 (1996-11), pages 6281-6287, XP000863110 ISSN: 0021-9193 the whole document --- -/--	1-27

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 February 2001	20. 2. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax (+31–70) 340–3016	Panzica, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00289

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BARENKAMP S J ET AL: "IDENTIFICATION OF A SECOND FAMILY OF HIGH-MOLECULAR-WEIGHT ADHESION PROTEINS EXPRESSED BY NON-TYPABLE HAEMOPHILUS INFLUENZAE" MOLECULAR MICROBIOLOGY,GB,BLACKWELL SCIENTIFIC, OXFORD, vol. 19, no. 6, 1996, pages 1215-1223, XP000579265 ISSN: 0950-382X the whole document	1-27
A	ST GEME III J W ET AL: "Prevalence and distribution of the hmw and hia genes and the HMW and Hia adhesins among genetically diverse strains of nontypeable Haemophilus influenzae" INFECTION AND IMMUNITY,US,AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, vol. 66, no. 1, January 1998 (1998-01), pages 364-368, XP002137980 ISSN: 0019-9567 the whole document	1-27
A	WO 96 02648 A (AMERICAN CYANAMID CO ;BACTEX INC (US); GREEN BRUCE A (US); BRINTON) 1 February 1996 (1996-02-01) the whole document	1-27
A	US 5 843 463 A (KRIVAN HOWARD C ET AL) 1 December 1998 (1998-12-01) the whole document	1-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 00/00289

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No. 23 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.24 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

2. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No. 25 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.26 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

3. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.27 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.28 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

4. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.29 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.30 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

5. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.31 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.32 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic

compositions containing the same.

6. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.33 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.34 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

7. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.35 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.36 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 00/00289

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9630519 A	03-10-1996	US 5646259 A	08-07-1997
		AU 718392 B	13-04-2000
		AU 5322896 A	16-10-1996
		CA 2216292 A	03-10-1996
		EP 0815236 A	07-01-1998
		JP 11502713 T	09-03-1999
WO 9602648 A	01-02-1996	US 5643725 A	01-07-1997
		US 5834187 A	10-11-1998
		US 5968769 A	19-10-1999
		AU 706937 B	01-07-1999
		AU 3097295 A	16-02-1996
		CA 2195090 A	01-02-1996
		EP 0771352 A	07-05-1997
US 5843463 A	01-12-1998	CA 2138765 A	06-01-1994
		EP 0647139 A	12-04-1995
		JP 2805174 B	30-09-1998
		JP 7509693 T	26-10-1995
		WO 9400149 A	06-01-1994
		US 5721115 A	24-02-1998
		US 5679547 A	21-10-1997
		AT 176989 T	15-03-1999
		CA 2098598 A	22-06-1992
		DE 69130955 D	08-04-1999
		DE 69130955 T	01-07-1999
		DK 565590 T	27-09-1999
		EP 0565590 A	20-10-1993
		ES 2131066 T	16-07-1999
		JP 6508346 T	22-09-1994
		WO 9210936 A	09-07-1992

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

STEWART, Michael I.
SIM & McBURNEY
330 University Avenue
6th Floor
Toronto, Ontario M5G 1R7
CANADA

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	17.07.2001
Applicant's or agent's file reference 1038-1025	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00289	16/03/2000	16/03/1999

Applicant
CONNAUGHT LABORATORIES LIMITED et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Neumann, M Tel.+49 89 2399-7351

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
1038-1025		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00289	16/03/2000	16/03/1999
International Patent Classification (IPC) or national classification and IPC		
C07K14/00		
Applicant		
CONNAUGHT LABORATORIES LIMITED et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report
   II ☐ Priority
   III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   IV ☒ Lack of unity of invention
   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   VI ☐ Certain documents cited
   VII ☐ Certain defects in the international application
   VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
11/10/2000	17.07.2001
Name and mailing address of the international preliminary examining authority:	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Zellner, E Telephone No. +49 89 2399 8427

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00289

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, pages:

1-13,16,17, 19-63	as originally filed			
14,15,18	as received on	22/06/2001	with letter of	22/06/2001

Claims, No.:

1-29	as received on	22/06/2001	with letter of	22/06/2001

Drawings, sheets:

1/83-83/83	as originally filed

Sequence listing part of the description, pages:

2-75, filed with the letter of 29.05.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT International application No. PCT/CA00/00289

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00289

Novelty (N)	Yes:	Claims 1-2,4-29
	No:	Claims 3
Inventive step (IS)	Yes:	Claims 4
	No:	Claims 1-3,5-29
Industrial applicability (IA)	Yes:	Claims 1-29
	No:	Claims

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/CA00/00289

D1: WO 96 30519 A (UNIV WASHINGTON ;UNIV ST LOUIS (US); ST GEME JOSEPH W III (US); BA) 3 October 1996 (1996-10-03)

D2: GEME J W S ET AL: 'CHARACTERIZATION OF THE GENETIC LOCUS ENCODING HAEMOPHILUS INFLUENZAE TYPE B SURFACE FIBRILS' JOURNAL OF BACTERIOLOGY,US,WASHINGTON, DC, vol. 178, no. 21, November 1996 (1996-11), pages 6281-6287, XP000863110 ISSN: 0021- 9193

D3: BARENKAMP S J ET AL: 'IDENTIFICATION OF A SECOND FAMILY OF HIGH-MOLECULAR-WEIGHT ADHESION PROTEINS EXPRESSED BY NON-TYPABLE HAEMOPHILUS INFLUENZAE' MOLECULAR MICROBIOLOGY,GB,BLACKWELL SCIENTIFIC, OXFORD, vol. 19, no. 6, 1996, pages 1215-1223, XP000579265 ISSN: 0950-382X

Item I

The amendment of Claim 3 is not allowable under Articles 19(2) and 34 (2) (b) PCT. Additional feature "N-truncated protein having the ability to bind to human epithelial cells" is not disclosed in the description as originally filed. For the N-truncated hia proteins it is only described that immunization causes protection against colonization (see Examples).

Item IV

The present set of claims are not linked in manner so as to form a single general inventive concept as required under Rule 13(1) PCT.
The problem underlying the invention of the present application is the provision of a set of nucleotide and amino acid sequences of adhesion (Hia) from non-typeable strains of Haemophilus influenzae.

The solution is represented by the set of amino- and nucleic acid sequences as set forth in SEQ. ID. Nos 23-36.

The international patent application WO9630519 discloses adhesins from non-typeable strains of Haemophilus influenzae, as well as methods for their recombinant production

and their use in immunogenic compositions and production of antibodies (see abstract, example 3, page 82-84).

Genes from non-typeable H. influenzae coding for Hia adhesins are also disclosed by St. Geme et al. in Infection and Immunity (1998, p. 364-368, see abstract).

Therefore the concept of DNA encoding adhesins from non typeable H. influenzae is not new. In consequence, the different adhesins of the present application fall a posteriori into 6 groups of alleged inventions.

1. Claims 1-27 (partially)
   An isolated and purified nucleic acid molecule having a sequence as set forth in SEQ ID NO. 23 encoding an polypeptide of a Haemophilus influenzae adhesion having the primary structure as set forth in SEQ ID NO. 24. Vectors for the recombinant production of said adhesion, immunogenic compositions containing the same.

2. An isolated and purified nucleic acid molecule having a sequence as set forth in SEQ ID NO. 27 encoding an polypeptide of a Haemophilus influenzae adhesion having the primary structure as set forth in SEQ ID NO. 28. Vectors for the recombinant production of said adhesion, immunogenic compositions containing the same.

3. An isolated and purified nucleic acid molecule having a sequence as set forth in SEQ ID NO. 29 encoding an polypeptide of a Haemophilus influenzae adhesion having the primary structure as set forth in SEQ ID NO. 30. Vectors for the recombinant production of said adhesion, immunogenic compositions containing the same.

4. An isolated and purified nucleic acid molecule having a sequence as set forth in SEQ ID NO. 31 encoding an polypeptide of a Haemophilus influenzae adhesion having the primary structure as set forth in SEQ ID NO. 32. Vectors for the recombinant production of said adhesion, immunogenic compositions containing the same.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/CA00/00289

5. An isolated and purified nucleic acid molecule having a sequence as set forth in SEQ ID NO. 33 encoding an polypeptide of a Haemophilus influenzae adhesion having the primary structure as set forth in SEQ ID NO. 34. Vectors for the recombinant production of said adhesion, immunogenic compositions containing the same.

6. An isolated and purified nucleic acid molecule having a sequence as set forth in SEQ ID NO. 35 encoding an polypeptide of a Haemophilus influenzae adhesion having the primary structure as set forth in SEQ ID NO. 36. Vectors for the recombinant production of said adhesion, immunogenic compositions containing the same.

Item V

1. Novelty:

Claim 3 is not allowable under Article 33 (2) PCT.
Due to the generic and broad definition (especially the wording "truncated" and "expressible" of said claim (see also Item VIII) all sorts of H. influenza adhesion encoding nucleotides fall under the definition of Claim 3.
In other words all adhesion nucleotides encoding for any adhesion being shorter (i.e. truncations of only one or two amino acids) than an adhesion of the present application lies within the definition such as those of D1 (see e.g. sequence comparisons of the Search Examiner page 6, in comparison with GSP:R99394 is shorter than no SEQ ID 28).

2. Inventive step

D1 is considered to represent the closest prior art document. D1 teaches Haemophilus adhesion proteins nucleic acids and derived vaccines. SEQ ID NO. 36 of the present application has 97% identity with the amino acid sequence of D1, SEQ ID NO 32 has 79% identity with the amino acid sequence of D1 (Sequence Comparisons of the Search Examiner).
The problem of the present application is to provide further H. influenza adhesions

proteins and their encoding genes. As soon as one family member of the Haemophilus influenza adhesion protein, its gene the recombinant production and its immunological use is known, it is routine for a skilled person to determine further similar members from other strains of said proteins their immunogenic fragments and their encoding genes.
In this case the cloning and expression, although requiring much work, does not pose such problems so that there was no reasonable expectation of success.
For a skilled person it is also obvious to provide non-specified truncated versions of said genes or proteins having no particular unexpected effect (Claim 3).

In consequence, the present claims 1-3, 5-29 are not allowable under Article 33 (3) PCT.

The specific truncated Hia proteins of Claim 4 fulfil the requirements under Article 33(2) and (3) PCT.

The essential difference with D1 is the truncated form wherein the signal sequence is deleted causing a higher expression in E. coli. Said truncated proteins are still immunogenic (see Examples).
The problem of the present application can thus be defined as the provision of alternative hia proteins which can be produced recombinantly in a high amount still causing immunity.
The solution i.e. the truncated hia proteins of claim 4 are not derivable from D1 or any other document cited in the Search Report.

Item VIII

1. Claim 2 is formulated in terms of a "product by process". In the PCT contracting states no unified criteria exist concerning this type of claims. Before the EPO such claims, defined in terms of a product by process of manufacture are only admissible if the product as such fulfils the requirements of patentability, i.e. if the products are novel and inventive and if the product cannot be defined by true technical features (Article 6 PCT).

   The same applies to claim 15.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/CA00/00289

2. Independent Claim 3 does not disclose any true technical features. The only characteristic of the claimed nucleic acids is that they are "truncated" and "expressible as inclusion bodies". In consequence, said claim is vague and thus not clear (Article 6 PCT).

3. The Applicant should prove whether the strains of Claim 27 are known by the skilled person. Otherwise said claim is not clear.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
1038-1025		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 00/00289	16/03/2000	16/03/1999
Applicant		
CONNAUGHT LABORATORIES LIMITED		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[X] furnished subsequently to this Authority in written form.

[X] furnished subsequently to this Authority in computer readble form.

[X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

[X] None of the figures.

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00289

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/285 C12N15/00 A61K38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, WPI Data, EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 30519 A (UNIV WASHINGTON ;UNIV ST LOUIS (US); ST GEME JOSEPH W III (US); BA) 3 October 1996 (1996-10-03) abstract example 3 page 82 -page 84	1-27
X	GEME J W S ET AL: "CHARACTERIZATION OF THE GENETIC LOCUS ENCODING HAEMOPHILUS INFLUENZAE TYPE B SURFACE FIBRILS" JOURNAL OF BACTERIOLOGY,US,WASHINGTON, DC, vol. 178, no. 21, November 1996 (1996-11), pages 6281-6287, XP000863110 ISSN: 0021-9193 the whole document	1-27

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 February 2001	20. 2. 01
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Panzica, G

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00289

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BARENKAMP S J ET AL: "IDENTIFICATION OF A SECOND FAMILY OF HIGH-MOLECULAR-WEIGHT ADHESION PROTEINS EXPRESSED BY NON-TYPABLE HAEMOPHILUS INFLUENZAE" MOLECULAR MICROBIOLOGY,GB,BLACKWELL SCIENTIFIC, OXFORD, vol. 19, no. 6, 1996, pages 1215-1223, XP000579265 ISSN: 0950-382X the whole document	1-27
A	ST GEME III J W ET AL: "Prevalence and distribution of the hmw and hia genes and the HMW and Hia adhesins among genetically diverse strains of nontypeable Haemophilus influenzae" INFECTION AND IMMUNITY,US,AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, vol. 66, no. 1, January 1998 (1998-01), pages 364-368, XP002137980 ISSN: 0019-9567 the whole document	1-27
A	WO 96 02648 A (AMERICAN CYANAMID CO ;BACTEX INC (US); GREEN BRUCE A (US); BRINTON) 1 February 1996 (1996-02-01) the whole document	1-27
A	US 5 843 463 A (KRIVAN HOWARD C ET AL) 1 December 1998 (1998-12-01) the whole document	1-27

International Application No. PCT/CA 00 00289

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No. 23 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.24 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

2. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No. 25 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.26 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

3. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.27 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.28 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

4. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.29 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.30 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

5. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.31 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.32 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

compositions containing the same.

6. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.33 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.34 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

7. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.35 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.36 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

International application No.
PCT/CA 00/00289

INTERNATIONAL SEARCH REPORT

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 00/00289

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9630519 A	03-10-1996	US 5646259 A	08-07-1997
		AU 718392 B	13-04-2000
		AU 5322896 A	16-10-1996
		CA 2216292 A	03-10-1996
		EP 0815236 A	07-01-1998
		JP 11502713 T	09-03-1999
WO 9602648 A	01-02-1996	US 5643725 A	01-07-1997
		US 5834187 A	10-11-1998
		US 5968769 A	19-10-1999
		AU 706937 B	01-07-1999
		AU 3097295 A	16-02-1996
		CA 2195090 A	01-02-1996
		EP 0771352 A	07-05-1997
US 5843463 A	01-12-1998	CA 2138765 A	06-01-1994
		EP 0647139 A	12-04-1995
		JP 2805174 B	30-09-1998
		JP 7509693 T	26-10-1995
		WO 9400149 A	06-01-1994
		US 5721115 A	24-02-1998
		US 5679547 A	21-10-1997
		AT 176989 T	15-03-1999
		CA 2098598 A	22-06-1992
		DE 69130955 D	08-04-1999
		DE 69130955 T	01-07-1999
		DK 565590 T	27-09-1999
		EP 0565590 A	20-10-1993
		ES 2131066 T	16-07-1999
		JP 6508346 T	22-09-1994
		WO 9210936 A	09-07-1992

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL SEARCHING AUTHORITY

To:
SIM & McBURNEY
Attn. Stewart, Michael, I.
330 University Avenue
6th Floor
Toronto, Ontario M5G 1R7
CANADA

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing (day/month/year) 20/02/2001
Applicant's or agent's file reference 1038-1025	FOR FURTHER ACTION See paragraph 2 below
International application No. PCT/CA 00/ 00289	International filing date (day/month/year) 16/03/2000
Applicant CONNAUGHT LABORATORIES LIMITED	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the International application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the International application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for International preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chantal Meyer

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments and any accompanying statement, under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the time of filing the amendments (and any statement) with the International Bureau, also file with the International Preliminary Examining Authority a copy of such amendments (and of any statement) and, where required, a translation of such amendments for the procedure before that Authority (see Rules 55.3(a) and 62.2, first sentence). For further information, see the Notes to the demand form (PCT/IPEA/401).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
1038-1025		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 00/ 00289	16/03/2000	16/03/1999
Applicant		
CONNAUGHT LABORATORIES LIMITED		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [X] furnished subsequently to this Authority in written form.

   [X] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00289

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/285 C12N15/00 A61K38/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, WPI Data, EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 30519 A (UNIV WASHINGTON ;UNIV ST LOUIS (US); ST GEME JOSEPH W III (US); BA) 3 October 1996 (1996-10-03) abstract example 3 page 82 -page 84 ---	1-27
X	GEME J W S ET AL: "CHARACTERIZATION OF THE GENETIC LOCUS ENCODING HAEMOPHILUS INFLUENZAE TYPE B SURFACE FIBRILS" JOURNAL OF BACTERIOLOGY,US,WASHINGTON, DC, vol. 178, no. 21, November 1996 (1996-11), pages 6281-6287, XP000863110 ISSN: 0021-9193 the whole document --- -/--	1-27

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 February 2001	20. 2. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Panzica, G

3

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BARENKAMP S J ET AL: "IDENTIFICATION OF A SECOND FAMILY OF HIGH-MOLECULAR-WEIGHT ADHESION PROTEINS EXPRESSED BY NON-TYPABLE HAEMOPHILUS INFLUENZAE" MOLECULAR MICROBIOLOGY,GB,BLACKWELL SCIENTIFIC, OXFORD, vol. 19, no. 6, 1996, pages 1215-1223, XP000579265 ISSN: 0950-382X the whole document	1-27
A	ST GEME III J W ET AL: "Prevalence and distribution of the hmw and hia genes and the HMW and Hia adhesins among genetically diverse strains of nontypeable Haemophilus influenzae" INFECTION AND IMMUNITY,US,AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, vol. 66, no. 1, January 1998 (1998-01), pages 364-368, XP002137980 ISSN: 0019-9567 the whole document	1-27
A	WO 96 02648 A (AMERICAN CYANAMID CO ;BACTEX INC (US); GREEN BRUCE A (US); BRINTON) 1 February 1996 (1996-02-01) the whole document	1-27
A	US 5 843 463 A (KRIVAN HOWARD C ET AL) 1 December 1998 (1998-12-01) the whole document	1-27

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No. 23 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.24 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

2. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No. 25 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.26 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

3. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.27 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.28 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

4. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.29 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.30 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

5. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.31 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.32 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

compositions containing the same.

6. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.33 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.34 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

7. Claims: 1-27 (in part)

An isolated and purified nucleic acid molecule having a sequence as set forth in Seq.Id.No.35 of the sequence listing, encoding for an aminoacid molecule of an Haemophilus influenzae adhesin having primary structure as set forth in Seq.Id.No.36 of the sequence listing. Vectors for the recombinant production of said adhesin, immunogenic compositions containing the same.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 00/00289

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 00/00289

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